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Leptin and the treatment of obesity: its current status

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Abstract

Leptin, the protein product of the *ob* gene, is primarily an adipocyte-secreted hormone, whose functional significance is rapidly expanding. Although early research efforts were focused on defining leptin's role in reversing obesity in rodents, there is now substantial evidence indicating that its influence extends to several hypothalamic–pituitary–endocrine axes, including gonadal, adrenal, thyroid, growth hormone, and pancreatic islets. A role for leptin in hematopoiesis, angiogenesis, immune function, osteogenesis, and wound healing has also been documented. The results of recent clinical trials with recombinant human leptin indicated that its effectiveness in restoring energy balance and correcting obesity-related endocrinopathies in genetically obese rodent models extended only partially to the management of human obesity. New efforts in drug development have focused on leptin-related synthetic peptide agonists as potential anti-obesity pharmacophores. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Studies utilizing spontaneous monogenic and transgenic mutant models of obesity in rodents have resulted in the identification of a number of proteins which play a role in regulating energy balance. Among these are leptin (OB), the leptin receptor (OB-R), agouti-related protein (AGRP), neuropeptide Y (NPY), carboxypeptidase E (Cpe), proopiomelanocortin (POMC), galanin, alpha-melanocyte stimulating hormone (α-MSH), the melanocortin 4-receptor (MC4-R), and cocaine- and amphetamine-regulated transcript (CART). The discovery of these proteins, and the physiological pathways in which they are involved, has contributed significantly to our understanding of the complexity of the regulatory mechanisms involved in maintaining energy homeostasis. Although caution must be taken in attempts to extrapolate the results of rodent studies to humans, the information provided by these studies has nonetheless suggested new targets for drug discovery which may have relevance to the treatment of human obesity.

This paper will provide an overview of one of these proteins, leptin. It will summarize what is currently known about the genetics, biochemistry, and biology of leptin and its receptor, describe the progress that has been made in the clinical application of leptin to human obesity, and explore the potential of leptin analogs and mimetics as alternative approaches to the pharmacological management of obesity in humans.

2. Leptin

2.1. Synthesis and secretion

Leptin, the protein product of the *ob* gene, exerts its influence on food intake, energy expenditure, body weight, and neuroendocrine function through actions on neuronal targets in the hypothalamus (Considine and Caro, 1997; Friedman and Halaas, 1998). Leptin is expressed primarily by white adipocytes in proportion to their size (Hamilton et al., 1995), and functions as the afferent signal in a negative feedback loop involved in the regulation of energy balance in rodents (Halaas et al., 1995; Pelleymounter et al., 1995). Leptin is also synthesized by the gastric epithelium, placental trophoblast, skeletal muscle, and mammary gland (Bado et al., 1998; Masuzaki et al., 1997; Wang et al., 1998a; Casabielle et al., 1997). Because there is strong correlation between plasma leptin concentrations and leptin mRNA levels in both humans and rodents (Considine et al., 1996a; Maffei et al., 1995), leptin secretion is characterized as

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constitutive, with no evidence of any significant storage in the adipocyte (Barr et al., 1997).

Leptin levels not only increase exponentially with increasing fat mass (Considine et al., 1996a), but also reflect states of energy imbalance, i.e., prolonged fasting significantly decreases plasma leptin while overfeeding increases it (Kolaczynski et al., 1996a). Changes in leptin levels in response to fasting and feeding, however, are disproportionate to changes in body weight or body fat mass, further supporting leptin's dual role as a marker of energy stores and a mediator of energy balance.

Leptin expression is regulated by a number of hormonal factors. In humans, leptin expression is directly correlated with insulin levels, increasing within several days after insulin infusion (Kolaczynski et al., 1996b), and declining when insulin levels fall during fasting (Boden et al., 1996). Glucocorticoids stimulate leptin synthesis in cultured adipocytes (Slieker et al., 1996), and leptin expression increases in response to chronic elevation of cortisol in humans (Cizza et al., 1997). Leptin synthesis is inhibited by testosterone (Blum et al., 1997), but not affected by ovarian sex steroids (Castracane et al., 1998). Administration of thyroid hormone reduces leptin levels in rodents (Escobar-Morreale et al., 1997), but there are conflicting reports regarding the interaction between leptin and the pituitary–thyroid axis in humans (Mantzoros et al., 1997a; Diekman et al., 1998).

Leptin synthesis is also stimulated by infection, endotoxin, and cytokines (Bornstein et al., 1998; Janik et al., 1997; Grunfeld et al., 1996). Exposure to cold (Trayhurn et al., 1996) and catecholamines have been shown to decrease leptin expression, probably through activation of β adrenergic receptors (Donahoo et al., 1997; Mantzoros et al., 1996). Cigarette smoking has also been associated with decreased leptin levels (Mantzoros et al., 1997b, 1998).

2.2. The ob gene

The mouse *ob* gene and its human homologue have been cloned (Zhang et al., 1994) and mapped to chromosomes 6 and 7q31.3, respectively (Zhang et al., 1994; Isse et al., 1995). The *ob* gene encodes a 4.5-kb mRNA transcript with a highly conserved 167-amino acid open reading frame. A 21-amino acid signal peptide is cleaved before release of mature leptin into the circulation. Leptin has been crystallized and its crystal structure suggests that it is a member of the cytokine family of hormones (Zhang et al., 1997; Madej et al., 1998). Human leptin is 84% identical to mouse leptin, and 83% identical to rat leptin (Ahima and Flier, 2000).

Mutation of the mouse *ob* gene results in a syndrome that includes obesity, increased body fat deposition, hyperglycemia, hyperinsulinemia, hypothermia, and impaired thyroid and reproductive function in both male and female homozygous *ob/ob* obese mice (Ingalis et al., 1950). Two distinct mutations of the *ob* gene have been identified. One mutant, SM/Ckc-+^{Dac}ob^{2J}/ob^{2J}, expresses no leptin mRNA (Lonnnqvist et al., 1995). The other, C57BL/6J, overexpresses by 20-fold a mRNA species resulting from a single-base mutation at codon 105 (Zhang et al., 1994). This mutation converts the coding sequence for arginine (Arg^{105}) in leptin to a premature stop codon, resulting in the production of a truncated mRNA for leptin, which is translated into a protein that appears to be degraded in the adipocyte. Administration of recombinant leptin to *ob/ob* mice, or to normal lean, or diet-induced obese mice results in weight loss through reduced food intake and increased energy expenditure (Pelleymounter et al., 1995; Weigle et al., 1995; Barash et al., 1996).

Expression of the *ob* gene in humans is highly correlated with body fat and body mass index, with greater expression observed in obese than in normal-weight individuals (Considine et al., 1996a). Because leptin concentrations are high in the serum of most obese humans, but decrease with weight loss, human obesity has been characterized as a disease caused by leptin resistance (Lonnnqvist et al., 1995; Misra and Garg, 1996). Furthermore, although plasma concentrations of leptin may be 5-fold higher in obese humans than non-obese individuals, cerebral spinal fluid levels are only modestly elevated in obesity, indicating that the rate-limiting factor contributing to leptin resistance in obese humans appears to be related to defective leptin transport into the central nervous system (Banks et al., 1996; Caro et al., 1996).

Although the *ob* gene is normal in most cases of human obesity, a frameshift mutation (Montague et al., 1997), and polymorphism in the 5′ untranslated region of the human *ob* gene (Considine et al., 1996b; Hager et al., 1998; Strobel et al., 1998) in a number of morbidly obese humans with low serum leptin concentrations have been reported. Possible linkage of extreme obesity to markers flanking the human *ob* gene has also been proposed (Clement et al., 1996; Reed et al., 1996). These findings suggest that administration of recombinant leptin, or leptin analogs or mimetics of even higher potency than leptin, may be possible approaches to the treatment of at least some forms of human obesity.

2.3. The OB-R gene

The leptin receptor (OB-R) gene was cloned from mouse choroid plexus and mapped to the diabetes (*db*) locus of mouse chromosome 4 (Tartaglia et al., 1995). Multiple transcripts of the leptin receptor, resulting from alternative splicing of OB-R mRNA, encode at least six OB-R isoforms (Lee et al., 1996; Wang et al., 1998b). All isoforms of the receptor share an identical extracellular domain at the amino terminus, but have cytoplasmic domains of different lengths arising from alternative RNA splicing at the most C-terminal coding exon. Five of the known receptor isoforms, OB-R_a, OB-R_b, OB-R_c, OB-R_d and OB-R_f, contain transmembrane domains. OB-R_e, lacking both transmembrane and cytoplasmic domains, circulates as a soluble receptor (Lee et al., 1996; Huang et al., 2001). Mouse and human leptin

receptor have similar amino acid sequences in both the extracellular (78% identity) and intracellular domains (71% identity) (Tartaglia et al., 1995; Chen et al., 1996).

Only OB-R_b, the long receptor isoform, contains motifs within its intracellular domain that are required for signal transduction. OB-R_b is a single membrane-spanning receptor that belongs to the class I family of cytokine receptors (Tartaglia et al., 1995). Leptin binding activates the Janus kinase (JAK)-signal transduction and activator of transcription (STAT) signaling cascade (Vaisse et al., 1996). Activation of OB-R_b, and to a lesser extent OB-R_a, also promotes JAK-dependent signaling to mitogen-activated protein kinase (MAPK) (Bjorbaek et al., 1997). Both OB-R_b and OB-R_a mediate leptin internalization via clathrin-coated pits, lysosomal degradation, and receptor downregulation (Uotani et al., 1999). Recent studies suggest that suppressor of cytokine signaling-3 (SOCS-3) also has a role in the leptin signaling pathway via its inhibition of leptin-induced tyrosine phosphorylation of JAK2 (Bjorbaek et al., 1999).

Defects in the leptin receptor produce a syndrome in the mutant diabetic *db/db* mouse that is phenotypically identical to the *ob/ob* mouse (Ghilardi et al., 1996). In contrast to *ob/ob* mice, however, administration of recombinant leptin to *db/db* mice does not result in reduced food intake and body weight (Roberts and Greenberg, 1996). In C57Bl/Ks *db/db* mice, a premature stop codon at the 3′-end of the OB-R_b transcript results in the synthesis of a receptor, OB-R_a, with a truncated intracellular domain. Lacking the necessary intracellular motifs, OB-R_a is unable to mediate JAK-STAT signaling (Ghilardi et al., 1996). Other mutations at the *db* locus resulting in transcripts lacking information for either transmembrane or intracellular domains of OB-R have also been identified (Friedman and Halaas, 1998; Li et al., 1998).

Although mutations of the leptin receptor are rare in humans, some cases of morbidly obese individuals with mutations in the *db* gene have recently been described (Clement et al., 1998; Oksanen et al., 1998). These mutations are expressed in a phenotype similar to that resulting from human *ob* gene mutations, i.e., characterized by hyperphagia, early onset obesity, hypogonadism, and impaired secretion of growth hormone and thyroid-stimulating hormone. Unlike *db/db* mice, however, the human *db* gene mutation is not associated with hyperglycemia and hypothermia (Clement et al., 1998).

2.4. Physiology of leptin

Although much attention has focused on the central effects of leptin on energy balance in rodents, there is now a significant body of evidence which suggests that leptin has a role in the regulation of several hypothalamic–pituitary–endocrine axes, i.e., gonadal, adrenal, thyroid, pancreatic islets, and growth hormone (Himms-Hagen, 1999). Leptin has also been implicated as having a role in hematopoiesis (Gainsford et al., 1996; Mikhail et al., 1997; Umemoto et al., 1997; Lacaud et al., 1998), angiogenesis (Sierra-Honigmann et al., 1998; Bouloumie et al., 1998), immune function (Stallone, 1994; Loffreda et al., 1998; Lee et al., 1999; Lord et al., 1998), osteogenesis (Iwaniec et al., 1998; Liu et al., 1997; Oerter-Klein et al., 1998; Thomas et al., 1999), and wound healing (Ring et al., 2000; Frank et al., 2000). The pleiotropic nature of leptin action has been the subject of a number of recent reviews (Considine, 1999; Himms-Hagen, 1999; Ahima and Flier, 2000; Harris, 2000).

2.4.1. Leptin and reproduction

The obese C57BL/6J *ob/ob* mouse has a genetically acquired form of hypogonadotropic hypogonadism (Swerdloff et al., 1976). Although as many as 20% of male *ob/ob* mice may be capable of reproducing if placed on a restricted diet, *ob/ob* females are invariably sterile, and thinning of *ob/ob* females to normal weight by dietary restriction does not correct their sterility (Lane and Dickie, 1954). Early sexual development is normal in *ob/ob* females; ovulation, however, does not occur and the mice remain prepubertal with no occurrence of estrous cycles (Swerdloff et al., 1976). Restoration of reproductive functions to *ob/ob* females has been shown to require delivery of hypothalamic extracts to the third ventricle (Batt, 1972), and administration of pituitary extracts (Smithberg and Runner, 1957), gonadotropins (Chehab et al., 1996), progesterone (Smithberg and Runner, 1965) and relaxin (Smithberg and Runner, 1957). These observations indicate that the infertility of *ob/ob* females is related to hypothalamic and pituitary hormone insufficiencies. The importance of leptin for maturation of the reproductive axis has been demonstrated by the ability of recombinant leptin to initiate puberty and restore fertility in homozygous *ob/ob* female mice and to accelerate the onset of puberty in wild type mice (Chehab et al., 1997; Ahima et al., 1997).

Leptin's involvement in human menarche, pregnancy, and lactation has been well documented (Matkovic et al., 1997; Masuzaki et al., 1997; Butte et al., 1997; Demarath et al., 1999; Kiess et al., 1999), and mutations of the *ob* and *db* genes have been shown to be associated with hypothalamic hypogonadism in humans (Strobel et al., 1998). Low leptin levels and absence of diurnal leptin rhythm accompany exercise-induced amenorrhea (Laughlin and Yen, 1997). Taken together, these observations suggest that leptin's role in reproduction may be to signal the adequacy of energy stores for reproductive function by influencing a number of target organs in the hypothalamic–pituitary–gonadal axis.

2.4.2. Leptin and hematopoiesis

Examination of leptin receptor distribution in hematopoietic tissue and stem cells during embryonic development strongly suggests a role for leptin in hematopoiesis (Chioffi et al., 1996; Gainsford et al., 1996; Bennett et al., 1996). Secretion of leptin by bone marrow adipocytes may provide a local source of leptin to precursor cells in addition to the leptin supplied by the plasma (Laharrague et al., 1998). These cells have been shown to respond to leptin in a dose-depend-

ent manner (Umemoto et al., 1997; Bennett et al., 1996; Chioffi et al., 1996). Recent studies indicate that leptin, together with other cytokines, influences the development of specific lineages of cells, particularly T cells and macrophages, very early in hematopoiesis (Gainsford et al., 1996; Mikhail et al., 1997; Umemoto et al., 1997; Lacaud et al., 1998). Direct effects of leptin on macrophage function have also been reported (Loffreda et al., 1998). A strong correlation between plasma leptin levels and white blood cell count has been observed in obese humans (Wilson et al., 1997).

2.4.3. Leptin and immune function

Impaired immune function has been noted in both *ob/ob* (Chandra, 1980) and *db/db* mice (Fernandes et al., 1978). These impairments have been observed principally in cell-mediated immune responses, in resistance to viral and bacterial infections, and in macrophage function (Stallone, 1994; Lee et al., 1999). Recent studies have related these abnormalities to what is now known about the lack of functional leptin or leptin receptors in these genetically obese mouse models. Leptin has been shown to act directly on $CD4^+$ helper T cells harvested from *ob/ob* mice, inducing their proliferation and increasing cytokine production (Lord et al., 1998). These helper cells express the long isoform of the leptin receptor. No effects of leptin were seen in similar experiments with $CD4^+$ helper T cells harvested from *db/db* mice which lack functional leptin receptor. These data strongly suggest a leptin receptor-mediated effect on T cell production and function.

2.4.4. Leptin and angiogenesis

A number of angiogenic factors have been identified in adipose tissue (Zhang et al., 1997), and recent evidence suggests that leptin should be included in this list (Cohen et al., 2001). Leptin receptors are present on human endothelial cells, and leptin has been shown to induce angiogenesis both in vitro and in vivo (Sierra-Honigmann et al., 1998; Bouloumie et al., 1998). It has been suggested that leptin may be a local regulator in the extensive remodeling of the vasculature that occurs physiologically in adipose tissue as its mass expands and contracts with the development and reversal of obesity (Crandall et al., 1997). The physiological cyclic angiogenesis and regression which occurs in the ovarian follicle may also be related to leptin action, since some leptin is synthesized and secreted by the ovary, and its release appears to be related to the time of ovulation (Riad-Garbiel et al., 1998).

2.4.5. Leptin and osteogenesis

The majority of studies in rodents and humans suggest that leptin enhances bone formation. The leptin-defective *fa/fa* rat has decreased bone mass, increased bone resorptive activity, and hypercalciuria that cannot be attributed to the diabetic condition of this animal model (Foldes et al., 1992). In vitro, leptin has been observed to increase the number of mineralized bone nodules in rat-derived bone marrow cultures (Iwaniec et al., 1998). These data are consistent with in vivo data which show that *ob/ob* mice respond to leptin treatment with increased osteoblast formation and bone deposition (Liu et al., 1997). In humans, leptin levels and obesity positively correlate with increased bone mass and rates of bone formation (Oerter-Klein et al., 1998).

While leptin's actions on bone may be mediated by direct effects on osteoblasts and osteoclasts, which are known to contain leptin receptors, indirect actions of leptin are also suggested. The effects of leptin on the hypothalamic–pituitary axis have been confirmed. Leptin has been shown to regulate the release of growth hormone and somatostatin, and to improve the body's response to insulin and insulin-like growth factor (Carro et al., 1997; Quintela et al., 1997). It is proposed that these may be mechanisms by which leptin indirectly influences homeostatic balance to favor bone formation rather than bone resorption. There is evidence to suggest that leptin-induced enhancement of osteoblast differentiation and suppression of adipocyte differentiation in human bone marrow may be responsible for the negative correlation between bone mineral density and body fat mass (Thomas et al., 1999).

3. Clinical trials with leptin

Because of the significant health risks associated with obesity, the primary medical goal of weight loss has become the reduction of co-morbidities. A large body of evidence indicates that a sustained loss of 5–10% of body weight can significantly reduce the risk for cardiovascular disease, diabetes, and mortality in obese individuals (Kopelman, 2000; Goodpaster et al., 1999; Williamson et al., 1995). Because of the palliative nature of currently available therapies for the management of obesity, however, the success of achieving long-term weight loss through behavior modification, i.e., diet and exercise, is limited. This inadequacy, coupled with an understanding of the epidemic proportions to which obesity among both children and adults has grown, the evolution of lifestyles which favor weight gain, and the association of obesity with increased morbidity and mortality, has contributed to an intensification of research efforts targeted toward the development new pharmacological approaches to the treatment of human obesity.

The recent withdrawal of fenfluramine and phentermine, two promising Food and Drug Administration (FDA)-approved appetite suppressants, from clinical use in the United States (Connolly et al., 1997) has provided an additional impetus for anti-obesity drug discovery. This action has left only two drugs currently available for long-term treatment of obesity. The first of these, sibutramine, suppresses appetite by altering norepinephrine and serotonin metabolism in the brain (Ryan et al., 1995). The other, orlistat, reduces fat absorption by inhibiting gastric, pancreatic and other gastrointestinal lipases (Guerciolini, 1997). Data from clinical trials with these drugs have been published (Jones et al., 1995; Hill

et al., 1999; Sjostrom et al., 1998; Davidson et al., 1999; Hollander et al., 1998), and indicate that both sibutramine and orlistat are of limited efficacy. These results support the notion that the complex and redundant physiologic pathways that defend humans against negative energy balance may make it impossible to treat human obesity effectively with any single pharmacologic approach. Obesity, as has been found for other chronic health conditions such as type 2 diabetes and hypertension, will more than likely require combination therapy for its management.

In humans, the relative long-term stability of body weight, the difficulty of sustaining intentional weight loss, and the behavioral and metabolic modifications that are associated with changes in body weight indicate that the deposition of body fat is a regulated process. The influences of energy reserves, which are stored as fat, on growth, puberty, fertility, and thyroid function have been well documented in rodents, and suggest that there may be signals reflecting adipose tissue mass that affect a number of neuroendocrine systems (Rosenbaum et al., 1997). In mice and rats, the protein product of the *ob* gene, leptin, has become a prime candidate for this role. The effectiveness of leptin in regulating body weight and food intake in rodent models of obesity has generated considerable interest in its possible application to the management of obesity in humans.

As indicated earlier in this review, a wealth of scientific information regarding the regulation of energy balance has become available since the discovery of leptin. Although most of this information was gathered from animal models of obesity, it has nonetheless provided some valuable insights toward our understanding of the physiological basis regulating energy balance in humans. Observational studies in humans indicate that leptin, as demonstrated experimentally in rodents and primates, is secreted primarily by adipocytes and appears to have a role in the control of body fat via central actions which regulate food intake, metabolism, the autonomic nervous system, and energy balance (Hukshorn et al., 2000).

The dramatic effects of leptin in leptin-deficient *ob/ob* mice raised expectations that human obesity might also be a state of leptin deficiency that could be corrected with exogenous leptin. This, however, has been shown not to be the case. In contrast to *ob/ob* mice, most obese individuals have elevated plasma leptin levels (Considine et al., 1996a) indicating that their obesity is the result of leptin resistance. Nonetheless, the possibility remained that therapeutic supplementation of circulating leptin by administration of exogenous leptin might be able to stimulate leptin signaling and action in obese individuals in much the same way as administration of exogenous insulin is used to manage the insulin resistance associated with type 2 diabetes mellitus.

This hypothesis has been tested in the clinic, and the results of two trials with recombinant methionyl human leptin have been published. A double-blind, placebo-controlled study examined the safety of leptin therapy in 73 obese subjects who self-administered a subcutaneous (s.c.) leptin injection daily for either four or 24 weeks (Heymsfield et al., 1999). A total of 54 lean individuals were in the control group. Throughout the study, lean subjects were maintained on a eucaloric diet, while obese subjects consumed a 500-kCal deficient diet. The results of this trial indicated that daily administration of recombinant methionyl human leptin induced modest dose-related weight loss in most but not all subjects, with a large degree of variability in the amount of weight lost by individual subjects. Injection site erythema was the only adverse side effect reported. Glycemic control during the course of treatment was unchanged. These results suggest that although the administration of high doses of exogenous leptin may reverse leptin insensitivity in some obese individuals, daily s.c. injection over an extended period of time is not easily tolerated.

A small number of obese humans with congenital leptin deficiency have been identified (Strobel et al., 1998; Montague et al., 1997). These individuals have a mutation in the *ob* gene, which is homologous to that in the *ob/ob* mouse. The results of a trial in which recombinant methyionyl human leptin was administered (s.c.) to one such individual, a 9-year-old leptin-deficient girl with severe early-onset obesity, have recently been published (Farooqi et al., 1999). In this study, daily treatment with leptin for 12 months resulted in sustained reduction in body weight, predominantly from a loss of body fat. The patient's total energy expenditure was similar before and after 12 months of therapy, with reduction in her basal metabolic rate counterbalanced by an increase in energy expenditure due to her increased physical activity. These findings indicated that the therapeutic effects of leptin in this child were primarily due to changes in energy intake.

Leptin gradually increased both basal and stimulated serum gonadotropin levels in this patient during the 12 months of treatment. At each evaluation, pelvic ultrasonography indicated a juvenile uterus and ovaries, and development of secondary sexual characteristics did not occur. After 12 months of treatment, however, the nocturnal pattern of gonadotropin release was pulsatile, suggesting an early onset of puberty.

A low titer of non-neutralizing antibodies was detected in the serum of this patient after 2 months of leptin therapy, and persisted throughout the course of the trial. Their presence resulted in a high serum concentration of leptin, and a delay in the peak concentration after leptin administration. Although these antibodies may have interfered with measurement of serum leptin, they apparently did not compromise the child's response to treatment, and could not be associated with any adverse effects. The authors report that the leptin injections were well tolerated, with no systemic or local reactions noted.

Thus, the clinical response to exogenously administered leptin in leptin-deficient humans is different from that in leptin-resistant obese humans. These data demonstrate that obese individuals who lack circulating leptin may be sensi-

tive to treatment with exogenous leptin, whereas the majority of obese humans, whose circulating leptin levels are higher than non-obese humans, show variable degrees of leptin resistance. Although leptin therapy may be effective in the management of some forms of human obesity, its efficacy appears to be limited by the varying degrees of leptin insensitivity found in most cases of human obesity.

The results of these initial trials in humans have generated a number of clinical questions regarding dose, route of administration, patient compliance, and potential side effects associated with prolonged leptin therapy. Nonetheless, they suggest a role for leptin, more than likely in combination with other therapies, in the management of at least some forms of human obesity. These initial results await confirmation in phase III clinical trials, which will evaluate the therapeutic efficacy of exogenous leptin administration in larger numbers of obese humans.

4. Leptin related agonists and obesity

Recent advances in our understanding of the molecular and physiological basis of energy balance have resulted in the identification of new targets for pharmacological regulation of body weight gain in humans. Currently receiving most attention are the melanocortin system (Spiegelman and Flier, 2001), neuropeptide Y (Zimanyi and Poindexter, 2000), the uncoupling proteins (Silva, 2000), and leptin (Walder and de Silva, 2001). Taken together, the results of these efforts suggest that the redundancy of systems which regulate energy balance in humans will more than likely significantly limit the efficacy of any single drug in the treatment of obesity. In a manner similar to hypertension, the preferred clinical approach to obesity management will probably include combination therapy with two or more drugs having different mechanisms of action, and influencing both energy intake and energy expenditure.

The limited clinical success of leptin has generated a great deal of interest in the development of leptin-related peptide agonists. One approach taken in these efforts has utilized the methodologies of solid phase peptide synthesis to construct peptide fragments corresponding to discrete domains within the leptin molecule, and to test the bioactivity of these fragment in rodent models. The earliest report (Martinez et al., 1996) and a follow-up study (Fruhbeck et al., 1998) using this approach describe the effects of pools of peptide fragments corresponding to the entire amino acid sequence of secreted rat leptin on body weight and thermoregulation in Wistar rats. Only one pool, containing five peptide fragments encompassing the domain between amino acids 127 and 167, had any significant effects on body weight and rectal temperature. These observations led the authors to conclude that the active site of rat leptin is localized toward the C-terminus of the molecule, between amino acid residues 127 and 167.

Although simple in design, the contribution of these studies to the search for new modulators of energy balance was significant. They were the first to demonstrate that the entire leptin molecule was not required for the expression, at least in part, of leptin's effects on body weight and thermogenesis. The results of these studies also indicated that peripherally administered synthetic peptides could induce weight loss in rodents, an event known to be mediated through a central effect of leptin in the hypothalamus.

The effects of central administration of leptin-related synthetic peptides has also been examined. In this study, three peptide fragments corresponding to sequences in human leptin, selected on the basis of predicted biological cleavage sites, were administered (i.c.v.) for 2 days into the lateral cerebral ventricle of adult Sprague–Dawley rats (Samson et al., 1996). Included among these peptides were a cyclized analog of a linear peptide corresponding to amino acid residues 116–167; a peptide corresponding to amino acid residues 57–92; and a peptide at the N-terminus of secreted human leptin corresponding to amino acid residues 22–56. Dose-related inhibition of food intake was observed only in response to the N-terminal peptide (22–56), but the effects of the active peptide on body weight gain could not be assessed within the time frame of the study. Based on these results, these investigators suggest that, in contrast to rat leptin, the activity of human leptin is at the N-terminus of the molecule, between amino acid residues 22 and 56.

It should be noted that these early studies were carried out in non-obese rodents with normal levels of endogenous leptin. The activity of the peptides in the presence of an obese phenotype was not examined. In 1997, the first report describing effects of leptin-related synthetic peptides on food intake and body weight gain in an obese animal model was published (Grasso et al., 1997). In this study, and in a number of follow-up reports from our laboratory (Grasso et al., 1999a; Rozhavskaya-Arena et al., 2000), the leptin-deficient C57BL/6J *ob/ob* mouse was used.

As indicated earlier in this review, the leptin deficiency seen in this animal model is caused by a single base mutation at codon 105 of the *ob* gene. This mutation is expressed as an mRNA species that encodes a truncated, inactive form of leptin (Zhang et al., 1994). Thus, we hypothesized that the activity of mouse leptin might be localized toward its C-terminus, in domains distal to amino acid 105. To test this hypothesis, six peptide amides corresponding to amino acids 106–167 of mouse leptin were synthesized, individually administered (i.p., 28 days) to female C57BL/6J *ob/ob* mice, and their effects on food intake and body weight gain assessed. Three of the peptides, encompassing the domain between amino acid residues 106 and 140, significantly reduced food intake and body weight gain, although to a lesser extent than reported for full-length leptin.

The decreased potency of the active peptides (approximately 30-fold lower on a molar basis than recombinant mouse leptin) was not unexpected, and suggested the possible presence of additional active sites toward the N-terminus of the molecule. To examine this possibility, 14 peptide amides encompassing the complete sequence of secreted mouse

leptin, were synthesized. Each peptide was individually administered (i.p., 7 days) to female *ob/ob* mice. As observed earlier (Grasso et al., 1997), only peptides corresponding to amino acid residues 106–120, 116–130, and 126–140 significantly reduced food intake and body weight gain (Grasso et al., 1999a). No activity was localized at the N-terminus of the molecule. The biological activity of one of these peptides (116–130), has been confirmed by other laboratories, using in vitro and in vivo systems, central and peripheral administration, and measuring biological endpoints other than food intake and body weight gain (Gonzalez et al., 1999; Malendowicz et al., 1999, 2000a,b; Tena-Sempere et al., 2000).

Our initial efforts to determine the mechanism of action of (116–130) produced some unexpected results. Competitive binding and signal transduction assays using cell lines transiently transfected with either the short or long isoform of the leptin receptor suggested that the observed effects of (116–130) on food intake and body weight gain in *ob/ob* mice (Grasso et al., 1997,1999a) might not be mediated by peptide binding and activation of leptin receptor (Grasso et al., 1999b). In that same study, further evidence supporting a mechanism of action independent of leptin receptor activation was provided by the ability of (116–130) to reduce food intake, body weight gain, and blood glucose levels in the *db/db* mouse, a hyperleptinemic rodent model of obesity genetically deficient in functional leptin receptor (Chen et al., 1996; Lee et al., 1996).

The majority of cases of human obesity are characterized by variable degrees of hyperleptinemia and leptin resistance (Friedman and Halaas, 1998). Thus, leptin-related analogs that restore energy balance and correct other metabolic abnormalities associated with the obese syndrome by way of signaling pathways distinct from those of leptin may have the potential to be even more effective than leptin in the management of human obesity. It is also possible that such analogs may be helpful in the treatment of obese humans whose disease is caused by mutation in the *ob* or *db* gene. In this light, the clinical significance of the results of our mechanism of action studies with (116–130) (Grasso et al., 1999b) may prove to be substantial.

The activity of (116–130) was recently localized to a restricted domain at its N-terminus, between amino acid residues 116 and 122 (Rozhavskaya-Arena et al., 2000). A synthetic peptide amide corresponding to this sequence has been named OB3 (patent pending). OB3 (1 mg/day, i.p., 7 days) significantly reduced food intake, body weight gain, and blood glucose levels in female C57BL/6J *ob/ob* mice (Rozhavskaya-Arena et al., 2000) and in female C57BLKS/J-m *db/db* mice (unpublished data).

We found that substitution of the leucine residue at position 4 of OB3 with its D-isomer significantly improved the potency of OB3 with respect to its anorexogenic action, effects on weight loss, and glycemic control (Rozhavskaya-Arena et al., 2000). Pair-feeding studies with this analog of OB3, [D-Leu^4]-OB3, indicated that its antihyperglycemic action was independent of its effects on caloric intake, and was associated with a concomitant reduction in serum insulin levels (Grasso et al., 2001). Taken together, the ability of [D-Leu^4]-OB3 to improve energy balance and glycemic control, as well as its anithyperinsulinemic action, all suggest that the clinical usefulness of this analog may extend beyond the treatment of human obesity to the therapeutic management of diabetes mellitus in the absence or presence of an obese background.

5. Conclusions

The World Health Organization has estimated that in 2035, approximately 300 million adults and children will be obese (WHO, 1997). Not unexpectedly, anti-obesity therapies that have been based on nutritional and behavioral modification alone have had only partial and temporary benefits, given the availability of plentiful quantities of energy-dense foods and lifestyles which have become more sedentary and less physically active. At the same time, existing pharmaceutical interventions have been shown to have only limited efficacy. Nonetheless, new insights into the molecular mechanisms underlying energy balance have numerous implications for the understanding and management of human obesity. Taken together, the data indicate that restoration of energy balance and reduction of co-morbidity and mortality in obese humans will probably not be achieved by drug therapy alone (Augustine and Rossi, 1999). The optimum benefits of pharmaceutical approaches to obesity management will require the support of permanent lifestyle changes which involve diet and exercise.

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